PATENT

Attorney Docket No. **FORS-03213**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Brow *et al.*
Serial No.: 08/520,946
Filed: 08/30/95
Entitled: **RAPID DETECTION AND IDENTIFICATION OF PATHOGENS**

Group No.: 1636
Examiner: W. Sandals

APPELLANTS' REPLY TO EXAMINER'S ANSWER

ATTN: Board of Patent Appeals and Interferences
Commissioner for Patents and Trademarks
Washington, D.C. 20231

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Dated: October 18, 1999

By: Marlene E. Garitano
Marlene E. Garitano

Sir:

This Reply is responsive to the Examiner's Answer (mailed August 18, 1999) to Appellants' Appeal Brief filed May 27, 1999. Appellants believe that the present Reply clearly demonstrates a failure of the Examiner's Answer to properly consider Appellants' Declaration, amendments, and arguments; to properly characterize and evaluate the teachings of the prior art; and to recognize the formal sufficiency of Appellants' Appeal Brief. Each issue is addressed in turn.

I. THE BRIEF CONTAINS APPROPRIATE REFERENCE TO RELATED APPEALS AND INTERFERENCES

The Examiner's Answer argues that Appellants' Appeal Brief "does not contain a statement identifying the related appeals and interference . . . it is presumed that there are none. The Board, however, may exercise its discretion to require an explicit statement as to the existence of any related appeals and interferences" (Examiner's Answer, page 3). Appellants must respectfully disagree and note that the following explicit statement was provided on page 3 of Appellants' Appeal Brief:

"II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to Appellant or to Appellant's legal representative."

II. CLAIMS 1 AND 44 SHOULD NOT BE GROUPED TOGETHER

The Examiner's Answer argues that "[t]he appellant's statement in the brief that certain claims do not stand or fall together is not agreed with because there is no argument provided for claims 1 and 44 . . . The open language of claim 1 embraces the limitation of manganese in the reaction, and therefore, claim 44 is a subset of claim 1 and the claims should be grouped together" (Examiner's Answer, page 4). Appellants must respectfully disagree. Independent Claim 1 specifies a method comprising providing an enzymatic cleavage means, a test nucleic acid substrate containing sequences derived from one or more microorganisms, and control cleavage products produced by cleavage of a reference nucleic acid derived from a microorganism; treating the test nucleic acid substrate under conditions such that the substrate forms one or more cleavage structures; reacting the cleavage means with the cleavage structures so that one or more test cleavage products are produced; and comparing the test cleavage products to the control cleavage products. Independent Claim 44 specifies that the enzymatic cleavage means is in a solution comprising manganese. The cited prior art references do not teach or suggest use of a manganese comprising solution for enzymatic cleavage means. Indeed, Lyamichev *et al.*, the only cited reference describing a cleavage agent, teaches buffers containing magnesium rather than manganese. Therefore, Claims 1 and 44 are separate independent claims with different limitations and must be considered independently.

III. THE EXAMINER HAS NOT ESTABLISHED *PRIMA FACIE* OBVIOUSNESS

A. Appellants' Claimed Invention

The following description of the presently claimed invention is provided to aid the Appeal Board in reviewing the case. Appellants provide descriptions of various elements of the present invention to help familiarize the Board with aspects of the invention. These description are provided simply to further an understanding of the invention and are not meant to limit the scope of the claims. Indeed, in describing certain aspects of the presently claimed

invention it is helpful to describe embodiments that may be of narrower scope than those encompassed by the claims.

Pending Claim 1 recites a method comprising providing an enzymatic cleavage means (an enzyme that is capable of cutting another molecule), a test nucleic acid substrate (a nucleic acid target [*e.g.*, DNA or RNA] that is to be cut by the enzymatic cleavage means and is provided as an experiment sample) containing sequences derived from one or more microorganisms (the nucleic acid is obtained from a microorganism), and control cleavage products produced by cleavage of a reference nucleic acid derived from a microorganisms (control samples made from the cutting of nucleic acid from a microorganism for use as a reference for comparison to experimental samples). The recited method steps comprise treating the test nucleic acid substrate under conditions such that the substrate forms one or more intra-strand secondary structures. Intra-stand secondary structures are regions of a single strand of nucleic acid that result from the folding of the single strand and the formation of chemical interactions between non-contiguous bases of the single strand (*See*, Specification, page 31, lines 7-10). This is in contrast to inter-strand structures formed by the interaction of two or more separate nucleic acid molecules (*e.g.*, a primer bound to a target nucleic acid) (*See*, Specification, page 31, lines 7-10). The claim further comprises the steps of reacting the cleavage means with the intra-strand secondary structures so that one or more test cleavage products are produced (the interaction of the cleavage means with the intra-strand secondary structure results in the cutting of the test nucleic acid to produce fragments [cleavage products]), and comparing the test cleavage products to the control cleavage products.

Claim 19 also requires nucleic acid with intra-strand secondary structure and cleavage of the intra-strand secondary structure to produce cleavage products. Claim 44 requires nucleic acid with intra-strand secondary structure, reacting an enzymatic cleavage means with the intra-strand secondary structure to produce cleavage products, and detecting the cleavage products.

Several elements of these claims are of particular relevance to the present appeal. Specifically, the claims all require that the target nucleic acid (*e.g.*, the test nucleic acid that is to be reacted with the cleavage means) contain **intra-strand secondary** structure. This secondary structure is reacted with the cleavage means to produce cleavage products. Claim

1 also requires the presence of **test** and **control** cleavage products which are compared. As discussed in detail below, the Examiner has failed to cite references that teach or suggest cleavage of **test** nucleic acids with **intra-strand** secondary structure or methods of comparing **test** cleavage products and **control** cleavage products resulting from such reactions.

B. The Examiner has not Established *Prima Facie* Obviousness

Appellants have provided detailed arguments in the Appeal Brief explaining the Examiner's failure to establish *prima facie* obviousness. The Examiner's Answer has failed to address and overcome these arguments and, in fact, has clearly established that the rejections should be withdrawn. Appellants herein highlight several independent reasons for withdrawal of the Examiner's rejections. Of particular relevance to this Appeal, Appellants herein demonstrate that the Examiner has improperly failed to consider the Declaration of Mary Ann D. Brow and has improperly failed to consider Appellants' amended claims and certain of Appellants' arguments. Furthermore, aspects of the Examiner's rejection are based on a complete mischaracterization of Barr *et al.* (BioTechniques 4:428 [1986])--a reference that teaches away from the presently claimed invention. Each of these issues is discussed briefly below.

1. The Cited References Teach Away from their Combination

The Examiner's Answer explains that the combination of the prior art references is based on the recitation of "PCR" in Lyamichev *et al.* and the fact that Young, Seela and Roling and Young *et al.* teach modification of PCR (Examiner's Answer, page 11). Appellants assert that this combination is improper and fails to establish *prima facie* obviousness because this combination would destroy the intended function of the references.

As discussed above, the presently claimed invention requires nucleic acids with **intra-strand secondary structure**. PCR involves the use of nucleic acids **without** intra-strand secondary structure (*e.g.*, the specification explains that PCR efficiency is limited by factors such as secondary structure [page 6, lines 11-15]).[1] Because the nucleic acids of the presently

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[1] *See also*, Promega Products & Applications, "Tip of the Week Archive: Optimizing PCR and RT-PCR" (Attached hereto at Tab 1) "Strong secondary structure in the template DNA may (continued...)

claimed invention have intra-stand secondary structure, they are not compatible with PCR. The Examiner has apparently ignored this required element of the presently claimed invention in constructing the combination. This type of combination is forbidden in an obviousness analysis. If a proposal for modifying the prior art in an effort to attain the claimed invention causes the art to become inoperable or destroys its intended function, then the requisite motivation to make the modification would not have existed. *See In re Fritch*, 972 F.2d 1260, 1265 n.12, 23 USPQ2d 1780, 1783 n.12 (Fed. Cir. 1992) ("A proposed modification [is] inappropriate for an obviousness inquiry when the modification render[s] the prior art reference inoperable for its intended purpose."); *In re Ratti*, 270 F.2d 810, 813, 123 USPQ 349, 352 (CCPA 1959) (holding the suggested combination of references improper under Section 103 because it "would require a substantial reconstruction and redesign of the elements shown in [a prior art reference] as well as a change in the basic principles under which [that reference's] construction was designed to operate"). In the Examiner's combination, the prior art references would be rendered inoperable for their intended purpose because the secondary structures of the present invention are not compatible with PCR. Thus, the Examiner has failed to establish *prima facie* obviousness and the Board should overrule the Examiner.

2. The Cited References Teach Away from the Examiner's Modification

Appellants brief argued that Lyamichev *et al.* teach away from the presently claimed invention because Lyamichev *et al.* teach that nucleic acids containing intra-stand secondary structure should not be used. Appellants also argued that the teachings of Lyamichev are limited to applications using cleavage of inter-strand secondary structures (*i.e.*, structures formed by multiple nucleic acids such as a target and a primer). The Examiner's Answer however, argues that Lyamichev *et al.* taught "that the enzyme could recognize and cleave intra-strand secondary structure" (Examiner's Answer, page 13).

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[1](...continued)
result in little or no amplification. To reduce the effects of secondary structure, add 7-deaza-dGTP . . . to the PCR reaction."

Appellants assert that the Examiner has applied the wrong standard of obviousness and has failed to apply the teachings of Lyamichev as a whole. The Examiner's argument is based on the idea that the enzyme of Lyamichev **could** be used to cleave intra-strand structures. However, the Examiner has not shown that Lyamichev, or any other reference, teaches that the enzyme **should** be used to cleave intra-strand structures. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the **desireability** of the combination (*In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 [Fed. Cir. 1990]). Indeed, Lyamichev very explicitly teaches that the enzyme **should not be used** to cleave intra-stand structures stating that inter-strand structures composed of a target and primer are used because the primer "greatly increases cleavage efficiency, and reduces the number of **unwanted cleavages at regions of secondary structure in the target nucleic acid**" (emphasis added). Thus Lyamichev *et al.* teach away from the cleavage of nucleic acids at regions of intra-strand secondary structure. Nothing in the other cited references makes up for this deficiency of Lyamichev as these references do not teach cleavage or intra-strand secondary structures (*See* Examiner's Answer, page 14 "Appellants have argued in Paper No. 17 that Young, Seela and Roling and Young et al. do not teach the cleavage of intra-strand secondary structures in nucleic acids. This is true."). It is impermissible to combine references when the references teach away from the combination (*See e.g.*, *In re Grasselli*, 713 F.2d 731, 218 USPQ 769, 779 [Fed. Cir. 1983]). Therefore the Examiner has failed to establish *prima facie* obviousness because the cited references, as a whole, teach away from the presently claimed invention.

a. The Examiner has Failed to Consider the Declaration of Mary Ann D. Brow

Even if, for the sake of argument, the Examiner had established *prima facie* obviousness, Appellants have clearly rebutted it with the Declaration of Mary Ann D. Brow (Tab D of Appellants' Appeal Brief). Mary Ann D. Brow is a co-author of Lyamichev *et al.* (*See*, paragraph 3) and explicitly states that "the experimental findings in Lyamichev *et al.* teach away from the use of intra-stand secondary structures as targets for cleavage means" (paragraph 4). The Declaration points to data in Lyamichev *et al.* showing inferior and **undesired** results when cleavage is conducted on nucleic acid with intra-strand secondary

structures (paragraphs 4-6). The Declaration summarizes the overall teaching of Lyamichev *et al.* with respect to intra-strand secondary structure, stating "Thus, in Lyamichev *et al.*, we taught that primers were needed for applications using the cleavage reactions and that cleavage of intra-strand secondary structures as targets in cleavage reactions was undesired" (paragraph 6).

Thus, a co-author of the Examiner's primary prior art reference (*i.e.*, Lyamichev *et al.*) explains that the reference teaches away from the presently claimed invention and provides a factual basis for this conclusion. The Examiner is not one skilled in the art and cannot counter Appellants' evidence without providing evidentiary support to the contrary (*See e.g.*, *In re Grose*, 592 F.2d 1161, 201 USPQ 57 [CCPA 1979]). The Examiner has provided none. Indeed, the Examiner has provided no challenge to Appellants' Declaration and has apparently failed to consider the Declaration of Mary Ann D. Brow with respect to DNA target nucleic acids. Although the Examiner has conceded that the prior art teaches away from the use of RNA targets (Examiner's Answer, pages 13-14), the Examiner has not considered the evidence with respect to DNA targets. It is error for the Examiner to have not considered the Declaration (*See e.g.*, *In Re Beattie*, 974 F.2d 1309, 1313, 24 USPQ2d 1040, 1042-43 [Fed. Cir. 1992]). Because there is no evidence in the record to counter the Declaration, the Board should overrule the Examiner..

b. The Examiner has Failed to Consider Appellants' Arguments

Appellants argued that the Examiner's combination of Seela and Roling with Lyamichev is improper because Seela and Roling and the state of the art teach away from the combination. Seela and Roling was cited by the Examiner to introduce the element of nucleotide analogues (*See*, Claims 6, 26, and 48). The Examiner cited Seela and Roling as teaching that the use of "nucleotide analogs helped protect the nucleic acids containing them from nuclease digestion, as well as reduce 'read through' problems frequently encountered in polymerase reactions," (Advisory Action, page 7). Appellants responded by arguing that Seela and Roling teach that incorporation of nucleotide analogs **protects from nucleic acids from cleavage with endonucleases**, teaching away from the use of nucleotide analogues in methods where cleavage is desired (*i.e.*, the methods of the presently claimed invention) (Appeal Brief, pages 18-19). Thus, Applicants provided evidence from the Seela and Roling

that explicitly teaches away from the presently claimed invention. The Examiner has completely ignored this evidence and has provided no response to Appellants' arguments. For this reason, the Board should overrule the Examiner.

c. The Examiner's Rejection is Based on a Substantial Mischaracterization of Barr *et al.*

Appellants provided further evidence that Seela and Roling cannot be combined with Lyamichev to yield the presently claimed invention because the state of the art (as demonstrated by Barr *et al.*, a reference that is co-authored by Seela, attached as Tab C to Appellants' Appeal Brief) teaches that nucleotide analogues disrupt secondary structure. The claims of the presently claimed invention require secondary structure. Thus, the state of the art teaches that the nucleotide analogues find no use with the presently claimed invention. The Examiner's Answer responded to this argument by completely mischaracterizing Barr *et al.* Specifically, the Examiner argued that:

> "Barr et al. . . . state '7-deazaguanine, by virtue of replacement of N-7 of the guanine ring by the methine moiety, precludes Hoogsteen bond formation (21). In contrast to inosine, however, Watson-Crick base pairing through the exocyclic amino group at the 2 position of the heterocycle is not impaired (21).' This statement in Barr et al. clearly sets forth the ability of nucleotide analogs in a single stranded sequence to form secondary structures as recited in the instant claimed invention. This does not 'teach away' from the invention." (Examiner's Answer, page 13).

The teaching in Barr *et al.* referred to by the Examiner does not support the Examiner's proposition. Specifically, folded secondary structures comprise at least two forms of interaction, including "Watson-Crick" base pairing to create regions of duplex and Hoogsteen bond formation, both contributing to the overall duplex stability. The elimination of Hoogsteen interactions through the use of nucleotide analogs as described by Barr *et al.* is for the express purpose of eliminating the secondary structures on which the presently claimed invention depends. This is made explicitly clear in column 2, page 431 of Barr *et al.*, stating:

"we would propose that this, and the observation that removal of nitrogen at the 7-position in **7-deazaguanine containing polymers precludes completely Hoogsteen bond formation** (21), are the most likely explanations for the favorable gel electrophoresis properties of polymers containing these analogs. Thus, **the combination of stable Watson-Crick base pairing capability and the inability to form higher secondary structures** make c_7dGTP an ideal reagent for the **structure independent** resolution of DNA sequence during the dideoxy method of sequence analysis." (emphasis added).

Barr *et al.* describes the now well established principle that 7-deazaguanine nucleotides analogues prevent the formation of secondary structure.[2] This reference, rather than making the use of these analogues in the presently claimed invention obvious, indicates that the cleavage of secondary structures in the nucleic acids containing these analogues is, in fact, an unexpected result. The cleavage of such nucleic acids is even more surprising when Barr is viewed in combination with the teachings of Seela and Roling, which teaches that these analogues serve to make nucleic acids resistant to endonucleases. Such a combination would suggest that, even if the suppression of secondary structure formation by the nucleotide analogue is not absolute, any structures that may form should be uncleavable by the cleavage means of the presently claimed invention.

Barr *et al.* teach the exact opposite conclusion than that relied upon by the Examiner. Therefore Appellants' evidence is unrebutted and the Examiner's combination of Seela and Roling and Lyamichev must be deemed improper. For the above reasons, the Board should overrule the Examiner.

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[2] *See*, Promega Products & Applications, "Tip of the Week Archive: Optimizing PCR and RT-PCR" (Attached hereto at Tab 1) "Strong secondary structure in the template DNA may result in little or no amplification. To reduce the effects of secondary structure, add 7-deaza-dGTP . . . to the PCR reaction."

3. The Examiner Failed to Consider Appellants' Amended Claims

Appellants' amended Claim 1 requires the cleavage of a test nucleic acid containing intra-stand secondary structure to generate a test cleavage product and the comparing of the test cleavage product to a control cleavage product. The Examiner's Answer has failed to cite references that teach or suggest all of the elements of amended Claim 1. Lyamichev *et al.* does not contemplate the cleavage of nucleic acid from a microorganism containing intra-strand secondary structure to generate a cleavage product, wherein the cleavage product is compared to a control cleavage product from a microorganism. There is simply no basis in Lyamichev for comparing cleavage products from any reaction, let alone from reactions involving intra-strand secondary structure, test and control samples, or nucleic acid from microorganisms. The Examiner's answer admits that Young, Seela and Roling, and Young *et al.* do not compensate for this deficiency because they "do not teach the cleavage of intra-strand secondary structures in nucleic acids" (Examiner's Answer, page 14). The Examiner argues that the supplemental references are simply provided to "demonstrate that the claimed limitations are merely adaptations of well known methods" (Examiner's Answer, page 14). However, none of the supplement references even remotely teach or suggest cleavage reactions (they relate to PCR or DNA sequencing--which, as discussed above, have nothing to do with and are not compatible with the structures of the presently claimed invention). Therefore, they cannot supply the missing elements of generating and comparing test and control **cleavage** products from microorganisms. Because the cite references do not teach or suggest all of the limitations of Appellants amended claims, *prima facie* obviousness has not been established and the Board should overrule the Examiner. (*See e.g.*, *In re Royka*, 490 F.2d 981, 180 USPQ 580 [CCPA 1974]).

CONCLUSION

Appellants have provided several independent bases for overturning the Examiner's single pending rejection. For the reasons set forth above, it is respectfully submitted that Appellants' claims should be passed to allowance.

Dated: October 18, 1999

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